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- (54) **HOST CELLS WITH ARTIFICIAL ENDOSYMBIONTS**
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(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**
U.S. PATENT DOCUMENTS

4,385,119 A	5/1983	Blakemore	
4,677,067 A	6/1987	Schwartz et al.	
6,004,815 A	12/1999	Portnoy et al.	
6,287,556 B1	9/2001	Portnoy et al.	
6,599,502 B2	7/2003	Portnoy et al.	
7,390,646 B2	6/2008	Andino-Pavlovsky et al.	
7,470,427 B2	12/2008	Cocking	
7,754,221 B2	7/2010	Szalay et al.	
8,021,662 B2	9/2011	Szalay et al.	
8,159,224 B2	4/2012	Fontius	
8,361,437 B2	1/2013	Sharma et al.	
8,406,498 B2	3/2013	Ortyn et al.	
8,828,681 B2	9/2014	Bell, III et al.	
8,859,281 B2 *	10/2014	Bell et al.	435/366
8,956,873 B2 *	2/2015	Bell et al.	435/455
9,023,612 B2 *	5/2015	Bell et al.	435/29
9,085,764 B2 *	7/2015	Bell et al.	435/29

2002/0012698 A1	1/2002	Bauerlein et al.
2004/0234455 A1	11/2004	Szalay
2005/0069491 A1	3/2005	Szalay et al.
2007/0202572 A1	8/2007	Szalay et al.
2007/0258886 A1	11/2007	Ahrens et al.
2007/0275924 A1	11/2007	Khan
2009/0181101 A1	7/2009	Rademacher et al.
2009/0311194 A1	12/2009	Hu et al.
2009/0325258 A1	12/2009	Matsunaga et al.
2010/0135912 A1	6/2010	Gambhir et al.
2010/0297022 A1	11/2010	Prato et al.
2011/0034753 A1	2/2011	Dobson et al.
2012/0021517 A1	1/2012	Jin et al.
2012/0184004 A1	7/2012	Kay et al.
2012/0184030 A1	7/2012	Dermanovic et al.
2012/0302819 A1	11/2012	Alphandery
2013/0183758 A1	7/2013	Bell, III et al.
2013/0224122 A1	8/2013	Gambhir
2013/0253303 A1	9/2013	Bell, III et al.
2013/0280173 A1	10/2013	Negev et al.
2014/0273203 A1	9/2014	Bell, III et al.
2015/0007359 A1	1/2015	Bell, III et al.
2015/0010937 A1	1/2015	Bell, III et al.
2015/0064787 A1	3/2015	Bell, III et al.

FOREIGN PATENT DOCUMENTS

EP	1807517 B1	10/2011
WO	WO 2013/106814	7/2013
WO	WO 2013/106814	7/2013
WO	WO 2014/145785	9/2014
WO	WO 2015/034962	3/2015

OTHER PUBLICATIONS

Wixon, Featured Organism: Reductive evolution in bacteria *Buchnera* sp. Rickettsia Prowazekii and . . . (2001) vol. 2, pp. 44-48.

Klasson et al., Horizontal gene transfer between Wolbachia and the mosquito *Aedes aegypti*, BMC Genomics (2009) vol. 10, pp. 33-42.

Iovieno et al., Detection of bacterial endosymbionts in clinical *acanthamoeba* isolates, (2010) Ophthalmology vol. 117, pp. 445-452.

Ordway et al., Animal models of mycobacteria infection, Current Protocols in Immunology (2011) Chapter 19, p. 19.5.1.

Goetz et al., Microinjection and growth of bacteria in the cytosol of mammalian host cells, 2001, Proc Natl Acad Sci vol. 98, pp. 12221-12226.

Bazylynski et al, Magnetosome formation in prokaryotes, 2004, Nature Reviews vol. 2, pp. 217-230.

Bell, Tracking cardiac engraftment and viability of MiPSC by MRI, 2014, Abstract for Grant Application, Project No. 1R43EB019239-01.

(Continued)

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(57) ABSTRACT

The present invention is directed generally to eukaryotic host cells comprising artificial endosymbionts and methods of introducing artificial endosymbionts into eukaryotic host cells. The invention provides artificial endosymbionts that introduce a phenotype to host cells that is maintained in daughter cells. The invention additionally provides eukaryotic host cells containing magnetotactic bacteria.

20 Claims, 3 Drawing Sheets

(56)

References Cited

OTHER PUBLICATIONS

- Brewer, et al., Relaxometry of Bacterially Derived Organelles: A Novel Class of MRI Contrast Agent for Cell Labeling and Tracking, May 2014, Joint Ann Mtg ISMRM-ESMRMB, Italy.
- Chen et al, Microwave breast tumor detection and size estimation using contrast-agent-loaded magnetotactic bacteria, 2013, 35th Ann Intl Conf IEEE EMBS Jul. 3, 2013, pp. 5781-5784.
- Frederickson et al, Inhibition of endosomal/lysosomal degradation increases the infectivity of HIV, 2002, J Virol vol. 76, pp. 11440-11446.
- Goldhawk et al, Using the magnetosome to model effective gene-based contrast for magnetic resonance imaging, 2012, WIREs Nanomed Nanobiotech vol. 4, pp. 378-388.
- Hautot et al, Preliminary observation of elevated levels of nanocrystalline iron oxide in the basal . . . 2007, Biochim Biophys Acta vol. 1772, pp. 21-25.
- Jogler et al, Genetic analysis of magnetosome biomineralization, 2006, Microb Monogr pp. 133-161.
- Katzmann et al, Loss of actin-like protein MamK has pleiotropic effects on magnetosome formation . . . 2010, Molc Microb vol. 77, pp. 208-224.
- Kirschvink et al., Biogenic magnetite as a basis for magnetic field detection in animals, 1981, Biosystems vol. 13, pp. 181-201.
- Kirschvink et al., Magnetite biomineralization in the human brain, 1992, Proc Natl Acad Sci vol. 89, pp. 7683-7687.
- Komeli et al., Molecular mechanisms of magnetosome formation, 2007, Ann. Rev. Biochem. vol. 76, pp. 351-366.
- Komeli, Molecular mechanisms of compartmentalization and biomineralization in magnetotactic bacteria, 2012, FEMS Microb Rev vol. 36, pp. 232-255.
- Mannucci et al., Magnetic nanoparticles from Magnetospirillum gryphiswaldense increase the efficacy of radiotherapy . . . 2014, PLoS One vol. 9, e108959.
- Matsunaga et al, Production of luciferase-magnetic particle complex by recombinant . . . AMB-1, 2000, Biotechnol Bioengin vol. 70, 704-709.
- Pradel et al, Biogenesis of actin-like bacterial cytoskeletal filaments destined for prokaryotic . . . 2006, Proc Natl Acad Sci vol. 103, pp. 17485-17489.
- Staniland et al., Controlled cobalt doping of magnetosomes in vivo, 2008, Nature Nanotechnol vol. 3, pp. 158-162.
- Tapper et al, Role of lysosomal and cytosolic pH in the regulation of macrophage lysosomal enzyme secretion, 1990, Biochem J vol. 272, pp. 407-414.
- Team:NYMU-Taipei-2011.igem.org screenshots of web-site.
- Tweten, Cholesterol-dependent cytolysins, a family of versatile pore-forming toxins, 2005, Infect Immun vol. 73, pp. 6199-6209.
- Vadia et al., the pore-forming toxin listeriolysin O mediates a novel entry pathway of *L. monocytogenes* into human hepatocytes, 2011, PLoS Pathog vol. 7, e1002356.
- Wakeel et al, an *Ehrlichia chaffeensis* tandem repeat protein interacts with multiple host targets in cell signaling . . . 2009, Infect Immun vol. 77, pp. 1734-1745.
- Wakeel et al, *Ehrlichia chaffeensis* tandem repeat proteins and Ank200 are type I secretion system substrates . . . 2011, Front Cell Infect Microb vol. 1, pp. 1-19.
- Zurkiya et al, MagA is sufficient for producing magnetic nanoparticles in mammalian cells, making it an MRI reporter, 2008, Magn Reson Med vol. 59, pp. 1225-1231.
- Re. 90/008,857, Oct. 7, 2008, Portnoy, Daniel A. et al.
- Re. 90/008,859, Sep. 30, 2008, Portnoy, Daniel A. et al.
- Re. 90/008,860, Oct. 14, 2008, Portnoy, Daniel A. et al.
- Agapakis, C.M. et al., Towards a synthetic chloroplast, PLoS One, Apr. 2011, 6: e18877.
- Ben-Halm, N. et al., Cell-specific integration of artificial organelles based on functionalized polymer vesicles, Nano Lett., 2008, 8:1368-1373.
- Benoit, M.R. et al., Visualizing implanted tumors in mice with magnetic resonance imaging using magnetotactic bacteria, Clin. Canc. Res., Aug. 15, 2009, 15(16):5170-5177.
- Benoit, M.R. et al., Supp. Figs., Clin. Canc. Res., 2009, at: http://clincancerres.aacrjournals.org/content/suppl/2009/08/11/1078-0432.CCR-08-3206.DC1/Supplementary_Data.pdf.
- Bernsen, M.R. et al., Labelling of mammalian cells for visualisation by MRI, Eur. Radial., 2010, 20: 255-274.
- Bhattacharya, D. et al., Photosynthetic eukaryotes unite: endosymbiosis connects the dots, BioEssays, 2003, 26:50-60.
- Bielecki, J. et al., *Bacillus subtilis* expressing a haemolysin gene from *Listeria monocytogenes* can grow in mammalian cells, Nature, May 10, 1990, 345:175-176.
- Bitterman, P.B. et al., Alveolar macrophage replication, J. Clin. Inv., Aug. 1984, 74: 460-469.
- Blakemore, R., Magnetotactic bacteria, Science, Oct. 24, 1975, 190(4212): 377-379.
- Blakemore, R.P. et al., Isolation and pure culture of a freshwater magnetic spirillum in chemically defined medium, J. Bacteriol. Nov. 1979, 140(2): 720-729.
- Bonnett, H.T., On the mechanism of the uptake of *Vaucheria* chloroplasts by carrot protoplasts treated with polyethylene glycol, Planta, 1976, 131: 229-233.
- Brown, M.B. et al., Exploiting tumour hypoxia in cancer treatment, Nat. Rev. Cancer, Jun. 2004, 4: 437-447.
- Budde, M.B. et al., Magnetic tagging of therapeutic cells for MRI, J. Nucl. Med., Feb. 2009, 50(2): 171-174.
- Bulte, J.W.M., In vivo MRI cell tracking: clinical studies, AJR Am. J. Roentgenol., Aug. 2009, 193(2): 314-325.
- Burdette, D.L. et al., *Vibrio* VopQ induces PI3-kinase-independent autophagy and antagonizes phagocytosis, Mol. Microbiol., 2009, 73(4): 639-649.
- Burgess, J.G. et al., Evolutionary relationships among Magnetospirillum strains inferred from phylogenetic analysis of 16S rDNA sequences, J. Bacteriol., 1993, 175: 6689-6694.
- Camilli, A. et al., *Listeria monocytogenes* mutants lacking phosphatidylinositol-specific phospholipase C are avirulent, J. Exp. Med., Mar. 1991, 173: 751-754.
- Cao, F. et al., In vivo visualization of embryonic stem cell survival, proliferation, and migration after cardiac delivery, Circulation, 2006, 113:1005-1014.
- Cheng, K. et al., Magnetic enhancement of cell retention, engraftment, and functional benefit . . . , Cell Transplant., 2012, 21: 1121-1135.
- Chico-Calero, I. et al., Hpt, a bacterial homolog of the microsomal glucose-6-phosphate translocase . . . , Proc. Natl Acad. Sci., Jan. 8, 2002, 99(1): 431-436.
- Chomel, B.B. et al., Ecological fitness and strategies of adaptation of *Bartonella* species to their hosts and vectors, Vet. Res., 2009, 40: 29.
- Cocking, E.C., et al., Symbiosome-like intracellular colonization of cereals and other crop plants by nitrogen-fixing bacteria . . . , Chin. Acad. Sci., 2005, 48: 888-896.
- Cocking, E.G., et al., Intracellular colonization of roots of arabidopsis and crop plants by *Gluconacetobacter diazotrophicus*, In Vitro. Cell. Dev. Biol., 2006, 42: 74-82.
- Concord, C. et al., Long-term evolutionary stability of bacterial endosymbiosis in Curculionioidea . . . , Mol. Biol. Evol., 2008, 25(5): 859-868.
- Corchero, J.L. et al., Biomedical applications of distally controlled magnetic nanoparticles, Trends Biotechnol., 2009, 27(8): 468-476.
- Dale, C. et al., The insect endosymbiont *Sodalis glossinidius* utilizes a type III secretion system for cell invasion, Proc. Natl Acad. Sci., Feb. 13, 2001, 98(4): 1883-1888.
- Dang, L.H., et al., Combination bacteriolytic therapy for the treatment of experimental tumors, Proc. Natl Acad. Sci., Dec. 18, 2001, 98(26): 15155-15160.
- Dubreuil, R. et al., Bringing host-cell takeover by pathogenic bacteria to center stage, Cell. Logist., 2011, 1(4): 120-124.
- Dyall, S.D. et al., Ancient invasions: from endosymbionts to organelles, Science, 2004, 304: 253-257.
- Faivre, D. et al., Magnetotactic bacteria and magnetosomes, Chem. Rev. 2008, 108: 4875-4898.

(56)

References Cited

OTHER PUBLICATIONS

- Felfoul, O. et al., MR imaging of Fe-Co nanoparticles, magnetotactic bacteria . . . , Proc. of the 7th IEEE International. Conf. on Nanotech., 2008, pp. 308-1.
- Finlay, B.B. et al., Common themes in microbial pathogenicity revisited, Microbiol. Mol. Biol. Rev., Jun. 1997, 61(2): 136-169.
- Fritsche, T.R. et al., Phylogenetic diversity among geographically dispersed Chlamydiales endosymbionts . . . , Appl. Environ. Microbiol., Jun. 2000, 66(6): 2613-2619.
- Goebel, W. et al., Intracellular survival strategies of mutualistic and parasitic prokaryotes, Trends Microbiol., Jun. 2001, 9(6): 267-273.
- Goetz, M. et al., Microinjection and growth of bacteria in the cytosol of mammalian host cells, Proc. Natl Acad. Sci., Oct. 9, 2001, 98(21): 12221-12226.
- Gordon, S. et al., Monocyte and macrophage heterogeneity, Nat. Rev., Dec. 2005, 5: 953-964.
- Gupta, R.S. et al., Phylogenomics and signature proteins for the alpha Proteobacteria and its main groups, BMC Microbiol., Nov. 28, 2007, 7:106.
- Hackam, D.J. et al., Rho is required for the initiation of Calcium signaling and phagocytosis by Fcγ receptors in macrophages, J. Exp. Med., Sep. 15, 1997, 186(6): 955-966.
- Hacker, H. et al., Caspase-9/-3 activation and apoptosis are induced in mouse macrophage upon ingestion and digestion of *E. coli* bacteria, J. Immunol., 2002, 169: 3172-3179.
- Hayward, R.D. et al., Direct nucleation and bundling of actin by the SipC protein of invasive *Salmonella*, EMBO J., 1999, 18(18): 4926-4934.
- Himmerle, U. et al., Stem cell labeling for magnetic resonance imaging, Min. Inv. Ther., 2008, 17:132-142.
- Hong, P.C. et al., Identification of genes required for chronic persistence of *Brucella abortus* in mice, Infect. Immun., Jul. 2000, 68(7): 4102-4107.
- Huang, J. et al., Phylogenomic evidence supports past endosymbiosis, intracellular and horizontal gene transfer in *Cryptosporidium parvum*, Genome Biol., Oct. 19, 2004, 5:R88.
- Joseph, B. et al., Identification of *Listeria monocytogenes* contributing to intracellular replication by expression profiling . . . , J. Bacteriol., Jan. 2006, 188(2): 556-68.
- Judas, M. et al., Genomic islands tools of bacterial horizontal gene transfer and evolution, FEMS Microbiol. Rev., 2009, 33: 376-393.
- Jutila, M.A. et al., Locally dividing macrophages in normal and inflamed mammary glands, Clin. Exp. Immunol., 1986, 66: 615-624.
- Kasinaskas, R.W. et al., *Salmonella typhimurium* lacking ribose chemoreceptors localize in tumor quiescence and induce apoptosis, Cancer Res., 2007, 67(7): 3201-3209.
- Kawaguchi, R. et al., Phylogeny and 16s rRNA sequence of *Magnetospirillum* sp. AMB-1, an aerobic magnetic bacterium, Nucleic Acids, Res., 1992, 20(5): 1140.
- Kimura, N.T. et al., Selective localization and growth of *Bifidobacterium bifidum* in mouse tumors following intravenous administration, Cancer Res., Jun. 1980, 40: 2061-2068.
- Kircher, M.F. et al., Noninvasive cell-tracking methods, Nat. Rev. Clin. Oncol., 2011, 8:677-688.
- Kraitchman, D.L. et al., Imaging of stem cells using MRI, Basic Res. Cardiol., 2008, 103: 105-113.
- Kraitchman, D.L. et al., Stem cell therapy: MRI guidance and monitoring, 2008, J. Magn. Reson. Imaging 27: 299-310.
- Lane, C.E. et al., The eukaryotic tree of life: endosymbiosis takes its TOL, Trends Ecol. Evol., 2008, 23(5): 268-275.
- Lang, C. et al., Expression of green fluorescent protein fused to magnetosome proteins in microaerophilic magnetotactic bacteria, Appl. Environ. Microbiol., 2008, 74: 4944-53.
- Lee, Z. et al., Imaging stem cell implant for cellular-based therapies, Exp. Biol. Med., 2008, 233: 930-940.
- Lemmon, M.J. et al., Anaerobic bacteria as a gene delivery system that is controlled by the tumor microenvironment, Gene Ther., 1997, 4: 791-796.
- Li, L.H. et al., Electrofusion between heterogeneous-sized mammalian cells in a pellet: potential applications in drug delivery . . . , Biophysical J., Jul. 1996, 71: 479-486.
- Liu, S. et al., Anticancer efficacy of systemically delivered anaerobic bacteria as gene therapy vectors targeting tumor hypoxia/necrosis, Gene Ther., 2002, 9: 291-296.
- Loessner, H. et al., Remote control of tumour-targeted *Salmonella enterica* serovar Typhimurium by the use of L-arabinose . . . , Cell. Microbiol., 2007, 9(6): 1529-1537.
- Login, F.H. et al., Antimicrobial peptides keep insect endosymbionts under control, Science, Oct. 21, 2011, 334: 362-365.
- Long, C.M. et al., In vivo tracking of cellular therapeutics using magnetic resonance imaging, Expert Opin. Biol. Ther., 2009, 9(3): 293-306.
- Long, M.E. et al., Disruption of *Francisella tularensis* schu S4 igil, iglJ, and pdpC genes results in attenuation . . . , Infect. Immun., Mar. 2013, 81(3): 850-861.
- Martel, S. et al., Towards MRI-controlled ferromagnetic and MC-1 magnetotactic bacterial carriers . . . , Proceed. 28th IEEE EMBS Ann. Intl Conf. NY, 2006, pp. 3399-3402.
- Matsunaga, T. et al., Phagocytosis of bacterial magnetite by leucocytes, Appl. Microbiol. Biotechnol., 1989, 31:401-405.
- Matsunaga, T. et al., Gene transfer in magnetic bacteria: transposon mutagenesis and cloning of genomic DNA fragments . . . , J. Bacteriol., May 1992, pp. 2748-2753.
- Matsunaga, T. et al., Complete genome sequence of the facultative anaerobic magnetotactic bacterium *Magnetospirillum* sp. strain AMB-1, DNA Res., 2005, 12: 157-166.
- Matsunaga, T. et al., Molecular bioengineering of bacterial magnetic particles . . . , Magnetoreception and Magnetosomes in Bacteria, 2006, pp. 227-54, Springer-Verlag, Berlin.
- Min, J. et al., Quantitative bioluminescence imaging of tumor-targeting bacteria in living animals, Nat. Protocols, 2008, 3(4): 629-636.
- Nishida, K. et al., Induction of biogenic magnetization and redox control by a component of the target of rapamycin complex 1 signaling pathway, PLoS Biol., 2012, 10:e1001269.
- Okamoto, N. et al., A secondary symbiosis in progress?, Science, Oct. 14, 2005, 310: 287.
- Oldroyd, G.E.D. et al., Reprogramming plant cells for endosymbiosis, Science, May 8, 2009, 324: 753-754.
- O'Riordan, M. et al., Innate recognition of bacteria by a macrophage cytosolic surveillance pathway, Proc. Natl Acad. Sci., Oct. 15, 2002, 99(21): 13861-13866.
- Pan, X. et al., Cationic lipid-coated magnetic nanoparticles associated with transferrin for gene delivery, Int. J. Pharm., Jun. 24, 2008, 358: 263-70.
- Pawelek, J.M. et al., Tumor-targeted *Salmonella* as a novel anti-cancer vector, Cancer Res., Oct. 15, 1997, 57: 4537-4544.
- Price, C.T.D. et al., Amoeba host-Legionella synchronization of amino acid auxotrophy and its role in bacterial adaptation . . . , Environ. Microbiol., 2013, 16(2): 350-358.
- Qiu, B. et al., Dual transfer of GFP gene and MGd into stem-progenitor cells, Acad. Radiol., 2010, 17: 547-552.
- Riegler, J. et al., Superparamagnetic iron oxide nanoparticle targeting of MSCs in vascular injury, Biomaterials, 2013, 34: 1987-1994.
- Rogers, W.J. et al., Technology insight: in vivo cell tracking by use of MRI, Nature Clin. Prac., Oct. 2006, 3(10): 554-562.
- Roodbeen R. et al., Synthetic cells and organelles: compartmentalization strategies, BioEssays, 2009, 31: 1299-1308.
- Salminen, M. et al., improvement in nuclear entry and transgene expression of baculoviruses by disintegration of microtubules . . . , J. Virol., Mar. 2005, 79(5): 2720-2728.
- Schaffer, D. et al., Biological imaging in animals with genetically encoded magnetic reporters, Jan. 30, 2013, at <http://www.berkeley.edu/news/2013/01/KeckAbstract.htm>.
- Scheffel, A. et al., The acidic repetitive domain of the *Magnetospirillum gryphiswaldense* MamJ protein . . . , J. Bacteriol., Sep. 2007, 189(17): 6437-6446.
- Schoen, P. et al., Gene transfer mediated by fusion protein hemagglutinin reconstituted in cationic lipid vesicles, Gene Ther., 1999, 6:823-832.

(56)

References Cited

OTHER PUBLICATIONS

- Schuler, D. et al., Dynamics of iron uptake and Fe₃O₄ biomineralization during aerobic and microaerobic growth . . . , J. Bacteriol., 1998, 180(1): 159-162.
- Schuler, D. et al., Bacterial magnetosomes: microbiology, biomineralization, and biotechnological applications, Appl. Microbiol. Biotechnol., 1999, 52: 464-473.
- Schultheiss, D. et al., Inactivation of the flagellin gene *flaA* in *Magnetospirillum gryphiswaldense* . . . , Appl. Environ. Microbiol., Jun. 2004, 70(6): 3624-2631.
- Shoemaker, R.H., The NCI60 human tumour cell line anticancer drug screen, Nature Rev., Oct. 2006, 6: 813-823.
- Silva, A.C. et al., Application of hyperthermia induced by superparamagnetic iron oxide nanoparticles in glioma treatment, Intl J. Nanomed., 2011, 6:591-603.
- Smith, G.A. et al., The two distinct phospholipases C of *Listeria monocytogenes* have overlapping roles . . . , Infect. Immun., Nov. 1995, 63(11): 4231-4237.
- Soghomonyan, S.A. et al., Positron emissions tomography (PET) imaging of tumor-localized *Salmonella* expressing HSV1-TK, Cancer Gene Ther., 2005, 12: 101-108.
- Stewart, F.J. et al., Lateral symbionts acquisition in a maternally transmitted chemosynthetic clam endosymbiosis, Mol. Biol. Evol., 2008, 25(4): 673-687.
- Sugimoto, Y. et al., Differential cell division history between neutrophils and macrophages, British J. of Haematology, 2006, 135: 725-731.
- Swiston, A.J. et al., Surface functionalization of living cells with multilayer patches, Nano Lett., 2008, 8(12): 4446-4453.
- Sznol, M. et al., Use of preferentially replicating bacteria for the treatment of cancer, J. Clin. Invest., Apr. 2000, 105(8): 1027-1030.
- Thao, M.L. et al., Phylogenetic analysis of vertically transmitted psyllid endosymbionts (*Candidatus Carsonella ruddi*) . . . , Curr. Microbiol., 2001, 42: 419-421.
- Vaishnav, S. et al., The cell biology of secondary endosymbiosis—how parasites build, divide and segregate the apicoplast, Mol. Microbiol., 2006, 61(6): 1380-1387.
- Valdivia R.H. et al., Endosymbiosis: the evil within, Current Biology, 2007, 17 (11): R408-R410.
- Van Der Glegen, M., Endosymbiosis: past and present, Heredity, 2005, 95: 335-336.
- Van Furth, R. et al., Dual origin of mouse spleen macrophages, J. Exp. Med., Nov. 1984, 160: 1273-1283.
- Verhoeven, H.A. et al., Direct cell to cell transfer of organelles by microinjection, Plant Cell Reports, 1992, 10: 613-616.
- Wajnberg, E. et al., Electron paramagnetic resonance study of the migratory ant *Pachycondyla marginate* abdomens, Biophys. J., Feb. 2000, 78: 1018-1023.
- Wennergren, J.J., Endosymbiosis: lessons in conflict resolution, PLoS Biology, Mar. 2004, 2(3): 307-311.
- Widermann, A. et al., *Yersinia enterocolitica* invasin triggers phagocytosis via B1 integrins, CDC42Hs and WASp in macrophages, Cellular Microbiol., 2001, 3(10): 693-702.
- Williams, K. et al., Proliferating cellular nuclear antigen expression as a marker of perivascular macrophages . . . , Am. J. Pathol., Aug. 2002, 161(2): 575-585.
- Wyss Institute, New “magnetic yeast” could be significant step in harnessing nature’s magnetic capabilities, Feb. 28, 2012, at <http://wyss.harvard.edu/viewpressrelease/78>.
- Xi, Z. et al., Characterization of *Wolbachia* transfection efficiency by using microinjection of embryonic cytoplasm . . . , Appl. Environ. Microbiol., 2005, 71(6): 3199-3204.
- Xie, J. et al., Production, modification, and bio-applications of magnetic nanoparticles gestated by Magnetotactic Bacteria, Nano Res., 2009, 2: 261-278.
- Yam, C. et al., Monotherapy with a tumor-targeting mutant of *S. typhimurium* inhibits liver metastasis in a mouse model . . . , J. Surgical Res., 2010, 164: 248-255.
- Yoshida, S. et al., *Shigella* deliver an effector protein to trigger host microtubule destabilization . . . , EMBO J., 2002, 21(12): 2923-2936.
- Zhang, X. et al., Artificial innate immune system . . . , Proc. of the 3rd Int’l Conf. on Artificial Immun. Sys. (ICARIS), LNCS 3239, 2004, pp. 302-315.
- Zhao, M. et al., Targeted therapy with a *Salmonella typhimurium* leucine-arginine auxotroph . . . , Cancer Res., Aug. 1, 2006, 66(15): 7647-7652.
- Zhao, M., et al., Monotherapy with a tumor-targeting mutant of *Salmonella typhimurium* . . . , Proc. Natl. Acad. Sci., Jun. 12, 2007, 104(24): 10170-10174.
- Murat, D. et al., The magnetosome membrane protein, MmsF is a major regulator of magnetite biomineralization in *Magnetospirillum* . . . AMB-1, Mol. Microb. 2012, 85:684-699.
- Lang, C. et al., Expression of green fluorescent protein fused to magnetosome proteins in microaerophilic magnetotactic bacteria, Appl. Environ. Microb. 2008, 74:4944-4953.
- Kovach, M.E., et al., Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes, Gene 1995, 166:175-176.
- U.S. Appl. No. 14/476,584, filed Sep. 3, 2014. Host Cell Modification with Artificial Endosymbionts.
- U.S. Appl. No. 14/489,316, filed Sep. 17, 2014. Host Cells with Artificial Endosymbionts.
- U.S. Appl. No. 14/582,823, filed Dec. 24, 2014. Host Cells with Artificial Endosymbionts.
- U.S. Appl. No. 14/689,987, filed Apr. 17, 2015. Eukaryotic Cells with Artificial Endosymbionts for Multimodal Detection.
- U.S. Appl. No. 14/732,911, filed Jun. 8, 2015. Host Cells with Artificial Endosymbionts.
- Bian et al., The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*, PLoS Pathogen vol. 6, p. e1000833 (2010).
- Calvitti, Bacteria endosymbionts: a source of innovation in biotechnology for the control of vector borne diseases, Energia Amb. Innovaz. n. 6, Nov-Div 2011, pp. 49-57.
- Yan et al, Magnetotactic bacteria, magnetosomes and their application, Microbiol. Res. (May 9, 2012) vol. 167, pp. 507-519.

* cited by examiner

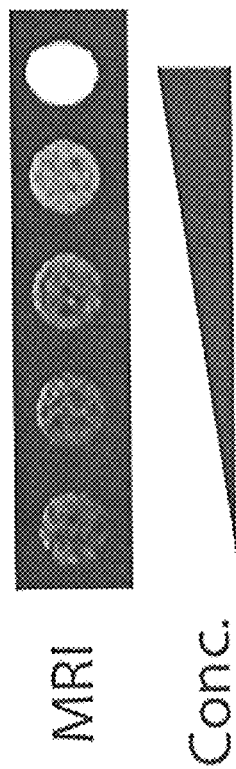
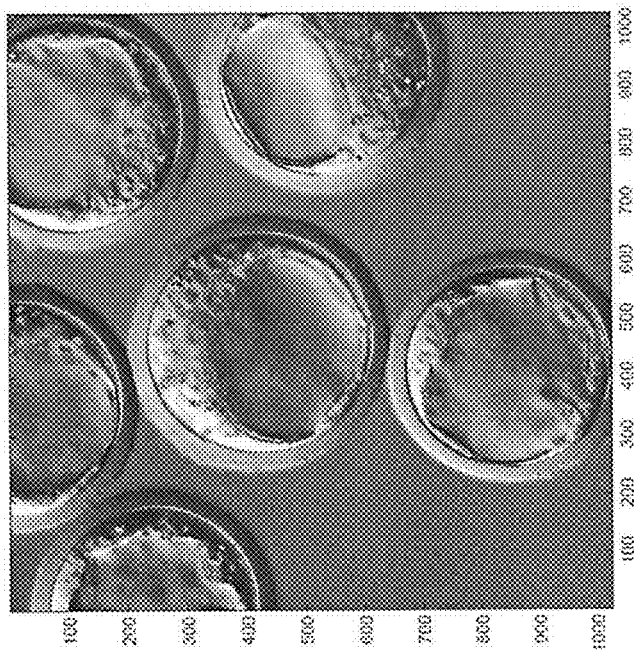
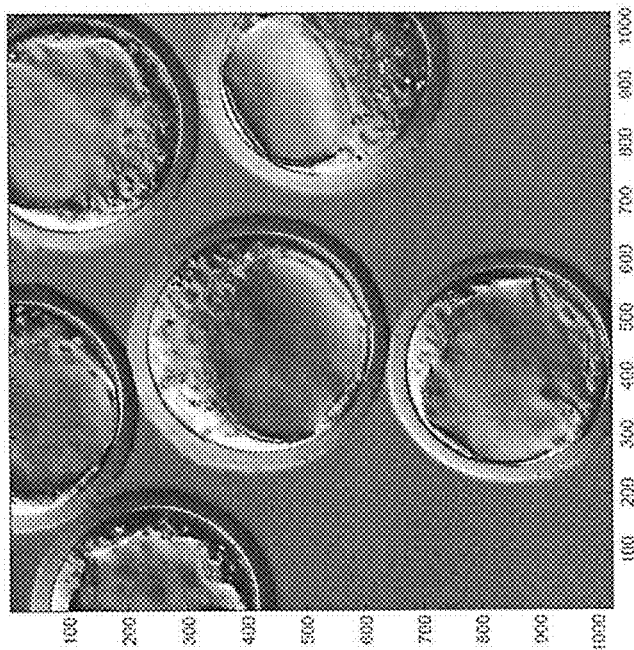


FIG. 1



Panel A



Panel B

FIG. 2

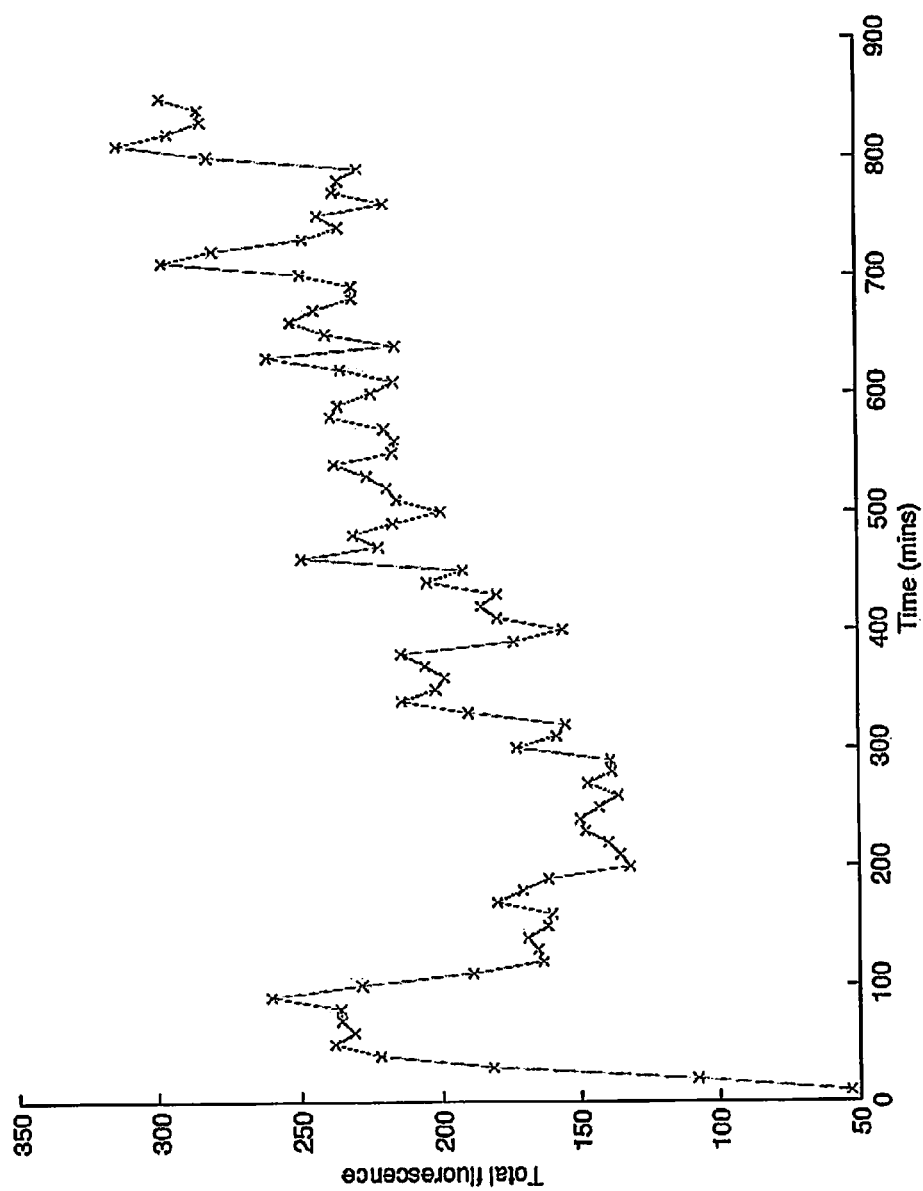


FIG. 3

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HOST CELLS WITH ARTIFICIAL ENDOSYMBIONTS

FIELD OF THE INVENTION

The present invention relates generally to the field of endosymbiosis, artificial endosymbionts, and magnetotactic bacteria. In particular, the invention provides artificial endosymbionts including magnetotactic bacteria, eukaryotic host cells for those artificial endosymbionts, and methods of introducing the artificial endosymbionts into the cytoplasm of host cells.

BACKGROUND OF THE INVENTION

Mitochondria, chloroplast and other membrane bound organelles add heritable functionalities, such as photosynthesis, to eukaryotic cells. Such organelles (identified by their vestigial circular DNA) are believed to be endosymbiotically derived.

Bacteria exist with a wide range of functionalities not present in various eukaryotic cells. For example, in 1975 Blakemore identified magnetotactic bacteria (MTB) that orient and swim along a geomagnetic field. (Blakemore, R. Magnetotactic bacteria. *Science* 24: 377-379 (1975) (which is incorporated by reference in its entirety for all purposes)). These magnetotactic bacteria produce magnetic structures called magnetosomes that are composed of magnetite (Fe_3O_4) or greigite (Fe_3S_4) enclosed by a lipid membrane. (Id.). A large number of MTB species have been identified since their initial discovery. (Id.).

Magnetotactic bacteria have been used to selectively bind to and separate substances. (U.S. Pat. No. 4,677,067 (which is incorporated by reference in its entirety for all purposes)). Additionally, attempts have been made to add magnetic functionality to cells through external tags. (Swiston, A. J., Cheng, C., Soong, H. U., Irvine, D. J., Cohen, R. J., Rubner, M. F. Surface Functionalization of Living Cells with Multilayer Patches. *Nano Lett.* 8(12): 4446-53 (2008) (which is incorporated by reference in its entirety for all purposes)). Bacterial magnetite has also been introduced into red blood cells by cell fusion (Matsunaga, T., Kamiya, S., (1988), In: Atsumi, K., Kotani, M., Ueno, S., Katila T., Williamsen, S. J. (eds) 6th International Conference on Biomagnetisms (1987). Tokyo Denki University Press, Tokyo, pp. 50-51 (which is incorporated by reference in its entirety for all purposes)), and MTB have been introduced into granulocytes and monocytes by phagocytosis. (Matsunaga, T., Hashimoto, K., Nakamura, N., Nakamura, K., Hashimoto, S. Phagocytosis of bacterial magnetite by leucocytes. *Applied Microbiology and Biotechnology* 31(4): 401-405 (1989) (which is incorporated by reference in its entirety for all purposes)). However, none of these alterations are heritable to daughter cells.

It is an object of the present invention to provide eukaryotic host cells containing artificial endosymbionts that are heritable to daughter cells. It is also an object of the present invention to provide methods of introducing artificial endosymbionts into the cytosol of eukaryotic host cells. It is another object of the present invention to provide eukaryotic host cells containing artificial endosymbionts that give the host cell a heritable magnetic phenotype.

SUMMARY OF THE INVENTION

The present invention relates to eukaryotic host cells comprising artificial endosymbionts and methods of intro-

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ducing artificial endosymbionts into eukaryotic host cells. In one embodiment, the artificial endosymbionts are heritable to daughter cells. In another embodiment, the artificial endosymbiont is a magnetotactic bacterium. The artificial endosymbiont of the invention may be modified by deleting, adding, and/or mutating at least one gene whereby the artificial endosymbiont acquires a trait useful for endosymbiosis or biotrophy. The genes to be mutated, added, and/or deleted in the artificial endosymbiont may be genes encoding components of the flagellar assembly and genes encoding enzymes for synthesizing essential macromolecules, such as amino acids, nucleotides, vitamins, and co-factors. In certain embodiments, the MTB may further be modified to express an antibiotic resistance gene or other selectable marker.

In some embodiments the host cells of the invention are mammalian, such as mouse, rat, rabbit, hamster, human, porcine, bovine, or canine. In another embodiment the artificial endosymbiont is transmitted from the host cell to daughter progeny host cells. In another embodiment, the method further comprises deleting, inserting, and/or mutating at least one gene from the eukaryotic host cell.

The artificial endosymbionts of the invention can be introduced into eukaryotic host cells by a number of methods known to those of skill in the art including, but not limited to, microinjection, natural phagocytosis induced phagocytosis, macropinocytosis, liposome fusion, erythrocyte ghost fusion, or electroporation.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows, positive contrast generated with a T_1 pulse sequence over a log scale concentration up to $\sim 10^8$ MTB/mL for gfp^+ AMB suspended in agar plugs using a 1.5 T instrument to optimize and characterize the imaging properties.

FIG. 2 shows a blastula stage mouse embryo that has had one of its two cells at the 2-cell embryo stage microinjected with gfp^+ AMB. The embryo is imaged with Leica SP2 AOBs spectral confocal inverted microscope surrounded by an environmental control chamber for live-cell imaging with 20x, 0.7 NA objective, and optical zoom of 3x. Panel A shows differential interference contrast (DIC) image and Panel B shows a gray scale fluorescence capture of the same image.

FIG. 3 shows the change of total embryo GFP fluorescence of four mouse embryos over time as measured by confocal microscopy. One of the two cells from the 2-cell stage of each embryo had been microinjected with gfp^+ AMB, and the total GFP fluorescence of each embryo was measured beginning at the 8-cell stage, 24 hours after microinjection.

DETAILED DESCRIPTION OF THE INVENTION

The invention is illustrated by way of example and not by way of limitation. It should be noted that references to "an" or "one" or "some" embodiment(s) in this disclosure are not necessarily to the same embodiment, and all such references mean at least one.

The present invention is directed to eukaryotic host cells containing artificial endosymbionts in the cytosol of the host cell, and methods of introducing artificial endosymbionts into the cytosol of the host cell. In one embodiment the artificial endosymbiont is genetically altered. In some embodiments the artificial endosymbionts are magnetotactic bacteria (MTB).

DEFINITIONS

As used herein, the term “AMB” refers to *Magnetospirillum magneticum* strain AMB-1.

As used herein, the term “artificial endosymbiont” refers to a single-celled organism that is or has been introduced into the cytosol of a eukaryotic cell through human intervention, which has been or can be transferred to daughter cells of the eukaryotic cell through at least five cell divisions, and which maintains sufficient copy number in the daughter cells so that a phenotype introduced by the artificial endosymbiont is maintained in the daughter cells.

As used herein, the term “cellular life cycle” refers to series of events involving the growth, replication, and division of a eukaryotic cell. It is divided into five stages, known as G₀, in which the cell is quiescent, G₁ and G₂, in which the cell increases in size, S, in which the cell duplicates its DNA, and M, in which the cell undergoes mitosis and divides.

As used herein, the term “cytosol” refers to the portion of the cytoplasm not within membrane-bound sub-structures of the cell.

As used herein, the term “daughter cell” refers to cells that are formed by the division of a cell.

As used herein, the term “essential molecule” refers to a molecule needed by a host cell for growth or survival.

As used herein, the term “genetically modified” refers to altering the DNA of a cell so that a desired property or characteristic of the cell is changed.

As used herein, the term “host cell” refers to a eukaryotic cell in which an artificial endosymbiont can reside.

As used herein, the term “liposome mediated” refers to artificial microscopic vesicles consisting of an aqueous core enclosed in one or more lipid layers, used to convey artificial endosymbionts to host cells.

As used herein, the term “magnetosome” refers to particles of magnetite (i.e., Fe₃O₄) or greigite (Fe₃S₄) enclosed by a sheath or membrane, either as individual particles or in chains of particles.

As used herein, the term “magnetotactic bacteria” or “MTB” refers to bacteria with genes encoding magnetosomes.

As used herein, the term “mammal” refers to warm-blooded vertebrate animals all of which possess hair and suckle their young.

As used herein, the term “microinjection” refers to the injection of artificial endosymbionts into host cells.

As used herein, the term “tagged artificial endosymbiont” refers to artificial endosymbionts that have a ligand on the surface of the endosymbiont.

As used herein, the term “parent cell” refers to a cell that divides to form two or more daughter cells.

As used herein, the term “receptor mediated” refers to a molecular structure or site on the surface of a host cell that binds with an artificial endosymbiont or a tagged artificial endosymbiont followed by internalization of the artificial endosymbiont.

Artificial Endosymbionts

Artificial endosymbionts of the invention include bacteria that are capable of surviving in a eukaryotic cell and maintain copy number such that the phenotype introduced by the endosymbiont is maintained in daughter cells. In some embodiments, the artificial endosymbionts do not kill the eukaryotic host cell without further human intervention. In some embodiments, the endosymbiont can stably maintain phenotype in the eukaryotic daughter cells through at least 3 cell divisions, or at least 4 division, or at least 5 divisions, or at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,

18, 19, or 20 cell divisions. In another embodiment, the endosymbiont can stably maintain phenotype in the eukaryotic daughter cells through 3-5 divisions, or 5-10 divisions, or 10-15 divisions, or 15-20 divisions.

In an embodiment of the invention, the artificial endosymbionts of the invention are genetically modified. Methods for genetically modifying bacteria are well known in the art. Typically, the bacteria will be genetically modified to improve their survival in eukaryotic host cells, and/or to reduce the toxicity of the endosymbiont to the eukaryotic host cell, and/or to provide the eukaryotic host cell with a useful phenotype. In one embodiment, the flagellar proteins of an endosymbiont are modified so that the endosymbiont no longer expresses the flagellar proteins in the eukaryotic host cell. In another embodiment, the endosymbiont is modified so that it can no longer synthesize an essential molecule that is preferably provided by the eukaryotic host cell. In an embodiment, the endosymbiont is genetically modified so that its cell cycle is coordinated with the cell cycle of the eukaryotic host cell so that copy number of the endosymbiont can be maintained at a sufficient level to impart the phenotype to daughter cells.

Embodiments of the invention include artificial endosymbionts that are *Proteobacteria*. Embodiments of the invention include artificial endosymbionts that are α -*Proteobacteria*. In the current taxonomic scheme based on 16S rRNA, α -*proteobacteria* are recognized as a Class within the phylum *Proteobacteria*, and are subdivided into 7 main subgroups or orders (Caulobacteriales, Rhizobiales, Rhodobacteriales, Rhodospirillales, Rickettsiales, Sphingomonadales and Parvularculales). (Gupta, R. S. Phylogenomics and signature proteins for the alpha *Proteobacteria* and its main groups. *BMC Microbiology*, 7:106 (2007) (which is incorporated by reference in its entirety for all purposes)).

A large number of α -proteobacterial genomes that cover all of the main groups within α -*proteobacteria* have been sequenced, providing information that can be used to identify unique sets of genes or proteins that are distinctive characteristics of various higher taxonomic groups (e.g. families, orders, etc.) within α -*proteobacteria*. (Id. (which is incorporated by reference in its entirety for all purposes)).

Embodiments of the invention include artificial endosymbionts that are magnetotactic bacteria (“MTB”). A large number of MTB species are known to those of ordinary skill in the art since their initial discovery in 1975 by Blakemore (Blakemore, R. Magnetotactic bacteria. *Science* 24: 377-379 (1975) (which is incorporated by reference in its entirety for all purposes)) and represent a group of microbes (Faivre, D. & Schüler, D. Magnetotactic bacteria and magnetosomes. *Chemistry Reviews* 108: 4875-4898 (2008) (which is incorporated by reference in its entirety for all purposes)). MTB have been identified in different subgroups of the *Proteobacteria* and the *Nitrospira* phylum with most of the phylotypes grouping in α -*Proteobacteria*. Currently, culturable MTB strains assigned as α -*Proteobacteria* by 16S rRNA sequence similarity include the strain originally isolated by Blakemore in 1975, *Magnetospirillum magnetotacticum* (formerly *Aquaspirillum magnetotacticum*), *M. gryphiswaldense*, *M. magneticum* strain AMB-1 (“AMB”), *M. polymorphum*, *Magnetospirillum* sp. MSM-4 and MSM-6, *Magnetococcus marinus*, marine vibrio strains MV-1 and MV-2, a marine spirillum strain MMS-1 and *Magnetococcus* sp. strain MC-1, as well as others. A number of MTB are available in pure culture, including AMB. The doubling time of AMB in pure culture is approximately eight hours and is close to that of a typical mammalian cell.

Standard MTB growth media uses succinic acid as the main carbon source, but MTB can be grown with fumarate, tartrate, malate, lactate, pyruvate, oxaloacetate, malonate, P-hydroxybutyrate and maleate as the sole carbon source. These metabolites are present inside eukaryotic cells. Microaerophilic, facultative anaerobic, and obligate anaerobic MTB strains have been identified. Oxygen concentrations in the cytosol of eukaryotic cells are low due to sequestration by proteins such as myoglobin and concentration in specific cellular locations, e.g., mitochondria, thus the microaerophilic or facultative anaerobic environment necessary for MTB growth is already present in the eukaryotic host cell.

MTBs can also be classified by the magnetic particles they synthesize, either magnetite (Fe_3O_4) or greigite (Fe_3S_4). Magnetite producers are microaerophilic or facultative anaerobic, need some oxygen source for magnetosome synthesis, and have optimal growth temperatures near physiological temperature.

In some embodiments, the artificial endosymbionts of the invention are genetically modified. Molecular biology tools have been developed for genetic manipulations of MTB most extensively in AMB and *M. gryphiswaldense* strain MSR-1 (reviewed in Jogler, C. and Schtiller, D. in Magnetoreception and Magnetosomes in Bacteria, New York, Springer, 2007 p 134-138 (which is incorporated by reference in its entirety for all purposes)). Since the genome of AMB was the first sequenced of any MTB, all MTB gene references herein refer to this genome unless otherwise specified. The genomes of two other *Magnetospirillum* strains and *Magnetococcus* sp. strain MC-1 have also been recently sequenced. Genes from these strains or other MTB strains, presently culturable or unculturable, sequenced or unsequenced, known or unknown, can be used in the present invention.

The genes responsible for magnetosome formation in MTB cluster in genomic islands, known as the magnetosome island (MAI). In *M. gryphiswaldense*, the 130 kb MAI is generally structured into four polycistronic operons: the mamAB operon has 17 identified ORFs extending over 16.4 kb; the mamGFDC operon has 4 identified ORFs is 2.1 kb and 15 kb upstream of mamAB; the mms6 operon has 6 identified ORFs is 3.6 kb and 368 bp upstream of the mamGFDC; the mamXY operon has 4 identified ORFs is located about 30 kb downstream of mamAB; and the monocistronic mamW gene. In the MAI the proteins: Mam W, Mgl457, Mgl458, Mgl459, Mms6, Mgl462, MamG, MamF, MamD, MamC, MamH, MamI, MamE, MamJ, MamK, MamL, MamM, MamN, MamO, MamP, MamA, MamQ, MamR, MamB, MamS, MamT, MamU and Mgl505 have been identified, many of which have been given specific functions in magnetosome formation. Four genes outside the MAI have been linked to magnetosome formation, mamY, mtaA, mmsF and mamX. Conserved MAI's have been found in other MTB with some differences in genomic organization and size.

In some embodiments, genetic modifications are made to the artificial endosymbiont. Such modifications can be directed modifications, random mutagenesis, or a combination thereof. Natural endosymbionts are donors of novel metabolic capabilities and derive nutritional requirements from the host.

Natural colonization of a host by the symbionts occurs in seven stages: 1) transmission, 2) entry, 3) countering of host defense, 4) positioning, 5) providing advantage to the host, 6) surviving in host environment and 7) regulation.

In some embodiments, mutual nutritional dependence (biotrophy) may be established between the artificial endosymbiont and the host cell. In one embodiment, the artificial endosymbiont comprises at least one deletion of a gene encoding an enzyme for synthesizing an essential molecule, wherein said essential molecule is produced by the eukaryotic host cell. An essential molecule can include, but is not limited to, an amino acid, a vitamin, a cofactor, and a nucleotide. For instance, biotrophy can be accomplished by knocking-out the ability of the artificial endosymbiont to make an amino acid, which will then be derived from the host. Glycine is a reasonable choice as it is highly abundant in mammalian cells and a terminal product in bacterial amino acid biogenesis; at least 22 other possibilities exist. The enzyme serine hydroxymethyltransferase converts serine into glycine at the terminus of the 3-phosphoglycerate biosynthetic pathway for amino acid production. In one embodiment, the artificial endosymbiont is an AMB in which the gene amb2339 (which encodes the enzyme serine hydroxymethyltransferase) is genetically modified. There are numerous methods for mutating or knocking-out genes known to those of ordinary skill in the art, including in vitro mutagenesis, targeted insertion of DNA into the gene of interest by homologous recombination or deletion of the gene (or operon, as most of the genes in the bacteria cluster in operons), or using endonucleases provided appropriate sites only around the target are present in the genome.

In another embodiment, nutritional dependence for an artificial endosymbiont on the host cell could also be established by eliminating the ability of the artificial endosymbiont to synthesize various metabolites, cofactors, vitamins, nucleotides, or other essential molecules.

In some embodiments of the invention, an MTB artificial endosymbiont has mutations and/or deletions in genes associated with motility and/or secretion. MTB are flagellated, and in some embodiments of the invention the MTB has a deletion and/or mutation in at least one gene encoding molecular machinery associated with the flagella, such that the magnetic bacterium does not produce a functional flagellum. Additionally, many MTB secrete various compounds, such as hydroxamate and catechol siderophores, which may be detrimental to or elicit an immune response from the host. In the sequenced genome of AMB, of the 4559 ORF's, 83 genes have been related to cell motility and secretion. The flagellar assembly is known to be composed of the gene products of amb0498, amb0500, amb0501, amb0502, amb0503, amb0504, amb0505, amb0610, amb0614, amb0615, amb0616, amb0617, amb0618, amb0619, amb0628, amb1289, amb1389, amb2558, amb2559, amb2578, amb2579, amb2856, amb3493, amb3494, amb3495, amb3496, amb3498, amb3824, and amb3827. The flagella is controlled by the chemotaxis machinery which is composed of at least the gene products of amb0322, amb0323, amb0324, amb0325, amb0326, amb1806, amb1963, amb1966, amb2333, amb2635, amb2640, amb2648, amb2652, amb2826, amb2932, amb3002, amb3003, amb3004, amb3007, amb3102, amb3329, amb3501, amb3502, amb3654, amb3879 and amb3880.

In one embodiment, genes encoding antibiotic resistance are inserted into the genome of the artificial endosymbiont. Host cells cultured in media containing the antibiotic will require the artificial endosymbiont for survival. Neomycin resistance is conferred by either one of two aminoglycoside phosphotransferase genes, which also provide resistance against geneticin (G418), a commonly used antibiotic for eukaryotes. Hygromycin B resistance is conferred by a

kinase that inactivates hygromycin B by phosphorylation. Puromycin is a commonly used antibiotic for mammalian cell culture and resistance is conferred by the *pac* gene encoding puromycin N-acetyl-transferase. External control of the antibiotic concentration allows intracellular regulation of the copy number of the artificial endosymbionts. Any other system where resistance or tolerance to an external factor is achieved by chemical modification of this factor can also be employed. An indirect nutritive advantage on host cells may also be established by using MTB artificial endosymbionts and a magnetic culture method. In this embodiment, magnetic fields are established to confer an advantage to cells with MTB artificial endosymbionts. This could be either by providing the means for attachment to culture matrix or the access to necessary growth or media factors.

In another embodiment, genetic modifications are made the MTB genome to enhance intracellular stability against the host defense mechanisms for a particular host cell type. Many eukaryote endosymbionts and endoparasites, such as the proteobacterial endosymbionts of insects such as *Buchnera*, *Wigglesworthia*, and *Wolbachia*; the methanogenic endosymbionts of anaerobic ciliates; the nitrogen-fixing symbionts in the diatom *Rhopalodia*; the chemosynthetic endosymbiont consortia of gutless tubeworms (*Olavius* or *Jnanidrilus*), the cyanobacterial endosymbionts of sponges, the endosymbionts of all five extant classes of *Echinodermata*, the *Rhizobia* endosymbionts of plants, various endosymbiotic algae, the *Legionella*-like X bacteria endosymbionts of *Ameoba proteus*, numerous *Salmonella* sp., *Mycobacterium tuberculosis*, *Legionella pneumophila*, etc. reside in membrane-bound vacuoles often termed symbiosomes, while some species, such as *Blochmannia*, the rickettsia, *Shigella*, enteroinvasive *Escherichia coli*, and *Listeria*, have the ability to inhabit the cytosol. The Dot-Icm Type IV secretory system is employed by many intracellular bacteria acquired by phagocytosis to evade the endocytic pathway and persist in the host cell. This system has been well-studied in *L. pneumophila* and consists of the proteins: DotA through DotP, DotU, DotV, IcmF, IcmQ through IcmT, IcmV, IcmW and IcmX. In *Photobacterium luminescens*, the luminescent endosymbiont of nematodes, the genes encoding RTX-like toxins, proteases, type III secretion system and iron uptake systems were shown to support intracellular stability and replication. The gene *bacA* and the regulatory system *BvrRS* are essential for maintenance of symbiosis between *Rhizobia* and plants as well as the survival of *Brucella abortus* in mammalian cells. The *PrfA* regulon enables some *Listeria* species to escape the phagosome and inhabit the cytosol. The desired cellular location (e.g., symbiosome or cytosol) of the intracellular MTB will dictate which genes are required to be expressed in the MTB (either directly from the genome or through a stable vector) for survival and proliferation in the host environment. The endogenous plasmid pMGT is highly stable in MTB and a number of other broad range vectors (those of *IncQ*, *IncP*, pBBR1, etc.) are capable of stable replication in MTB.

In another embodiment, the artificial endosymbiont is genetically modified by knocking in genes, such as bacteriostatic gene(s), siderophore gene(s), metabolic requirement gene(s), suicide gene(s), life cycle regulation gene(s), transporter gene(s), and escape from the phagosome gene(s). In another embodiment, the artificial endosymbionts are randomly mutated and subsequently screened for enhanced integration within the host cell. Random mutation can be

accomplished by treatment with mutagenic compounds, exposure to UV-light or other methods known to those skilled in the art.

In another embodiment, transgenetic modification(s) are made to counter host cell defenses using genes from various parasites or endosymbionts. In another embodiment, the population of the artificial endosymbionts in the host cell cytosol is regulated through a balance of intrinsic use of host mechanisms (nutrient availability, control of reproduction, etc.) and antibiotic concentration.

In another embodiment, a natural endosymbiont or an intracellular parasite is genetically modified into an artificial endosymbiont to produce magnetosomes. Endosymbionts of insects such as *Buchnera*, *Wigglesworthia*, and *Wolbachia*; the methanogenic endosymbionts of anaerobic ciliates; the nitrogen-fixing symbionts in the diatom *Rhopalodia*; the chemosynthetic endosymbiont consortia of gutless tubeworms (*Olavius* or *Inanidrilus*), the cyanobacterial endosymbionts of sponges, the endosymbionts of all five extant classes of *Echinodermata*, the *Rhizobia* endosymbionts of plants, various endosymbiotic algae, the *Legionella*-like X bacteria endosymbionts of *Ameoba proteus*, numerous *Salmonella* sp., *Mycobacterium tuberculosis*, *Legionella pneumophila* belong to α -proteobacteria and could be genetically engineered to produce magnetosomes. In another embodiment, a pre-existing organelle can be genetically modified to express one or more magnetosome genes to produce an artificial endosymbiont. For instance, mitochondria, plastids, hydrogenosomes, apicoplasts or other organelles, which harbor their own genetic material, can be genetically altered.

In a preferred embodiment, the artificial endosymbiont is an MTB, which may or may not be genetically altered, that produces magnetic particles upon culturing of the eukaryotic host cells.

Eukaryotic Host Cells

The invention provides eukaryotic host cells comprising artificial endosymbionts in the host cells and methods of introducing the artificial endosymbionts into host cells. In some embodiments the host cells of the invention are mammalian, such as mouse, rat, rabbit, hamster, human, porcine, bovine, or canine. Mice routinely function as a model for other mammals, most particularly for humans. (See, e.g., Hanna, J., Wernig, M., Markoulaki, S., Sun, C., Meissner, A., Cassady, J. P., Beard, C., Brambrink, T., Wu, L., Townes, T. M., Jaenisch, R. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* 318: 1920-1923 (2007); Holtzman, D. M., Bales, K. R., Wu, S., Bhat, P., Parsadanian, M., Fagan, A., Chang, L. K., Sun, Y., Paul, S. M. Expression of human apolipoprotein E reduces amyloid- β deposition in a mouse model of Alzheimer's disease. *J. Clin. Invest.* 103(6): R15-R21 (1999); Warren, R. S., Yuan, H., Matli, M. R., Gillett, N. A., Ferrara, N. Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis. *J. Clin. Invest.* 95: 1789-1797 (1995) (each of these three publications is incorporated by reference in its entirety for all purposes)).

In some embodiments, the host cell is a human cancer cell. There are many human cancer cell lines that are well known to those of ordinary skill in the art, including common epithelial tumor cell lines such as Coco-2, MDA-MB231 and MCF7, non-epithelial tumor cell lines, such as HT-1080 and HL60, the NCI60-cell line panel (see, e.g., Shoemaker, R., The NCI60 human tumour cell line anticancer drug screen. *Nature Reviews Cancer* 6, 813-823 (2006) (which is incorporated by reference in its entirety for all

purposes)). Additionally, those of ordinary skill in the art are familiar with obtaining cancer cells from primary human tumors.

In other embodiments, the host cells are stem cells. Those of ordinary skill in the art are familiar with a variety of stem cell types, including Embryonic Stem Cells, Inducible Pluripotent Stem Cells, Hematopoietic Stem Cells, Neural Stem Cells, Epidermal Neural Crest Stem Cells, Mammary Stem Cells, Intestinal Stem Cells, Mesenchymal stem cells, Olfactory adult stem cells, and Testicular cells.

In an embodiment, the eukaryotic host cell is a cell found in the circulatory system of a human host. For example, red blood cells, platelets, plasma cells, T-cells, natural killer cells, or the like, and precursor cells of the same. As a group, these cells are defined to be circulating host cells of the invention. The present invention may be used with any of these circulating cells. In an embodiment, the eukaryotic host cell is a T-cell. In another embodiment, the host cell is a B-cell. In an embodiment the eukaryotic host cell is a neutrophil. In an embodiment, the eukaryotic host cell is a megakaryocyte.

In another embodiment, at least one gene from the eukaryotic host cell is genetically altered. In some embodiments, mutual nutritional dependence (biotrophy) may be established between the artificial endosymbiont and the host cell by genetic modification of the host cell, using the appropriate molecular biology techniques specific to the target host cell type known to those of ordinary skill in the art, creating host dependence on the artificial endosymbiont for some essential macromolecule thus establishing the environmental pressures for biotrophy. In another embodiment, nutritional dependence for an artificial endosymbiont on the host cell may be established by genetically altering the host cell to eliminate the ability of the artificial endosymbiont to synthesize various metabolites, cofactors, vitamins, nucleotides, or other essential molecules. In such embodiments, the essential molecule may be provided by the artificial endosymbionts. In another embodiment, the eukaryotic host cell gene encoding the enzyme serine hydroxymethyltransferase, which converts serine into glycine at the terminus of the 3-phosphoglycerate biosynthetic pathway for amino acid production, may be modified.

Methods of Introducing Artificial Endosymbionts to Host Cells

The artificial endosymbionts of the invention can be introduced into eukaryotic host cells by a number of methods known to those of skill in the art including, but not limited to, microinjection, natural phagocytosis, induced phagocytosis, macropinocytosis, liposome fusion, erythrocyte ghost fusion, electroporation, receptor mediated methods, and the like. (See Microinjection and Organelle Transplantation Techniques, Celis et al. Eds.; Academic Press: New York, 1986 and references therein, (incorporated by reference in its entirety for all purposes)).

In one embodiment, the artificial endosymbiont is introduced to the host cell by microinjection into the cytoplasm of the host cell. A variety of microinjection techniques are known to those skilled in the art. Microinjection is the most efficient of transfer techniques available (essentially 100%) and has no cell type restrictions (Id.; Xi, Z. & Dobson, S. Characterization of *Wolbachia* transfection efficiency by using microinjection of embryonic cytoplasm and embryo homogenate. *Appl. Environ. Microbiol.* 71(6): 3199-3204 (2005); Goetz, M., Bubert, A., Wang, G., Chico-Calero, I., Vazquez-Boland, J. A., Beck, M., Slaghuis, J., Szalay, A. A., Goebel, W. Microinjection and growth of bacteria in the cytosol of mammalian host cells. *Proc. Natl. Acad. Sci. USA*

98:12221-12226 (2001) (each of these three publications is incorporated by reference in its entirety for all purposes)).

Naturally phagocytotic cells have been shown to take up bacteria, including MTB (Burdette, D. L., Seemann, J., Orth, K. *Vibrio* VopQ induces PI3-kinase independent autophagy and antagonizes phagocytosis. *Molecular microbiology* 73: 639 (2009); Wiedemann, A., Linder, S., Grassi, G., Albert, M., Autenrieth, I., Aepfelbacher, M. *Yersinia enterocolitica* invasin triggers phagocytosis via $\beta 1$ integrins, CDC42Hs and WASp in macrophages. *Cellular Microbiology* 3: 693 (2001); Hackam, D. J., Rotstein, O. D., Schreiber, A., Zhang, W., Grinstein, S. Rho is required for the initiation of calcium signaling and phagocytosis by Fc γ receptors in macrophages. *J. of Exp. Med.* 186(6): 955-966 (1997); Matsunaga, T., Hashimoto, K., Nakamura, N., Nakamura, K., Hashimoto, S. Phagocytosis of bacterial magnetite by leucocytes. *Applied Microbiology and Biotechnology* 31(4): 401-405 (1989) (each of these our publications is incorporated by reference in its entirety for all purposes)).

This method is scalable, but may be limited to specific cell types (e.g., macrophage). However, recent studies have shown that non-phagocytotic cell types can be induced to endocytose bacteria when co-cultured with various factors: media and chemical factors, biologic factors (e.g., baculovirus, protein factors, genetic knock-ins, etc.). (See, e.g., Salminen, M., Airene, K. J., Rinnankoski, R., Reimari, J., Valilehto, O., Rinne, J., Suikkanen, S., Kukkonen, S., Yla-Herttuala, S., Kulomaa, M. S., Vihinen-Ranta, M. Improvement in nuclear entry and transgene expression of baculoviruses by disintegration of microtubules in human hepatocytes. *J. Virol.* 79(5): 2720-2728 (2005); Modalsli, K. R., Mikalsen, S., Bukholm, G., Degre, M. Microinjection of Hep-2 cells with coxsackie B1 virus RNA enhances invasiveness of *Shigella flexneri* only after prestimulation with UV-inactivated virus. *APMIS* 101: 602-606 (1993); Hayward, R. D. & Koronakis, V. Direct nucleation and bundling of actin by the SipC protein of invasive *Salmonella*. *The EMBO Journal* 18: 4926-4934 (1999); Yoshida, S., Katayama, E., Kuwae, A., Mimuro, H., Suzuki, T., Sasakawa, C. *Shigella* deliver an effector protein to trigger host microtubule destabilization, which promotes Rac 1 activity and efficient bacterial internalization. *The EMBO Journal* 21: 2923-2935 (2002); Bigildeev et al. *J. Exp. Hematol.*, 39: 187 (2011); Finlay, B. B. & Falkow, S. Common themes in microbial pathogenicity revisited. *Microbiol. and Mol. Biol. Rev.* 61: 136-169 (1997) (each of these six publications is incorporated by reference in its entirety for all purposes).

The related process, macropinocytosis or "cell drinking," is a method numerous bacteria and viruses employ for intracellular entry (Zhang (2004) In: Molecular Imaging and Contrast Agent Database (MICAD) [database online]; Bethesda (Md.): National Library of Medicine (US), NCBI; 2004-2011 (each of these two publications is incorporated by reference in its entirety for all purposes)). Various protocols exist which can be employed to induce cells to take up bacteria.

Several agents, such as nucleic acids, proteins, drugs and organelles have been encapsulated in liposomes and delivered to cells (Ben-Haim, N., Broz, P., Marsch, S., Meier, W., Hunziker, P. Cell-specific integration of artificial organelles based on functionalized polymer vesicles. *Nano Lett.* 8(5): 1368-1373 (2008); Lian, W., Chang, C., Chen, Y., Dao, R., Luo, Y., Chien, J., Hsieh, S., Lin, C. Intracellular delivery can be achieved by bombarding cells or tissues with accelerated molecules or bacteria without the need for carrier particles. *Experimental Cell Research* 313(1): 53-64 (2007);

Heng, B. C. & Cao, T. Immunoliposome-mediated delivery of neomycin phosphotransferase for the lineage-specific selection of differentiated/committed stem cell progenies: Potential advantages over transfection with marker genes, fluorescence-activated and magnetic affinity cell-sorting. *Med Hypotheses* 65(2): 334-336 (2005); Potrykus (1990) Ciba Found Symp, Vol. 1 54: 198 (each of these four publications is incorporated by reference in its entirety for all purposes)). This method is inexpensive, relatively simple and scalable. Additionally, liposome uptake can be enhanced by manipulation of incubation conditions, variation of liposome charge, receptor mediation, and magnetic enhancement. (See, e.g.,

Pan et al. *Int. J. Pharm.* 358: 263 (2008); Sarbolouki, M. N. & Toliat, T. Storage stability of stabilized MLV and REV liposomes containing sodium methotrexate (aqueous & lyophilized). *J. Pharm. Sci. Technol.*, 52(10): 23-27 (1998); Elorza, B., Elorza, M. A., Sainz, M. C., Chantres, J. R. Comparison of particle size and encapsulation parameters of three liposomal preparations. *J. Microencapsul.* 10(2): 237-248 (1993); Mykhaylyk, O., Sánchez-Antequera, Y., Vlaskou, D., Hammerschmid, E., Anton, M., Zelphati, O. and Plank, C. Liposomal Magnetofection. *Methods Mol. Bio.*, 605: 487-525 (2010) (each of these four publications is incorporated by reference in its entirety for all purposes)).

Erythrocyte-mediated transfer is similar to liposome fusion and has been shown to have high efficiency and efficacy across all cell types tested (Microinjection and Organelle Transplantation Techniques; Celis et al. Eds.; Academic Press: New York, 1986 (which is incorporated by reference in its entirety for all purposes)). Typically erythrocytes are loaded by osmotic shock methods or electroporation methods (Schoen, P., Chonn, A., Cullis, P. R., Wilschut, J., and Schuerrer, P. Gene transfer mediated by fusion protein hemagglutinin reconstituted in cationic lipid vesicles. *Gene Therapy* 6: 823-832 (1999); Li, L. H., Hensen, M. L., Zhao, Y. L., Hui, S. W. Electrofusion between heterogeneous-sized mammalian cells in a pellet: potential applications in drug delivery and hybridoma formation. *Biophysical Journal* 71:479-486 (1996); Carruthers, A., & Melchior, D. L. A rapid method of reconstituting human erythrocyte sugar transport proteins. *Biochem.* 23: 2712-2718 (1984) (each of these three publications is incorporated by reference in its entirety for all purposes). Alternatively, erythrocytes may be loaded indirectly by loading hematopoietic progenitors with artificial endosymbionts and inducing them to differentiate and expand into erythrocytes containing artificial endosymbionts.

Electroporation is a commonly used, inexpensive method to deliver factors to cells. (Potrykus, I. Gene transfer methods for plants and cell cultures. *Ciba Found Symp* 154, 198-208; discussion 208-112 (1990); Wolbank, S. et al. Labelling of human adipose-derived stem cells for non-invasive in vivo cell tracking. *Cell Tissue Bank* 8, 163-177 (2007) (each of these two publications is incorporated by reference in its entirety for all purposes)).

In another embodiment, a eukaryotic host cell that naturally endocytoses bacteria (e.g., Chinese hamster ovary (CHO)) is used. In one embodiment, the modified artificial endosymbionts are added to the CHO culture directly. CHO cells are cultured by standard procedures in Ham's F-12 media with 10% fetal calf serum media prior to infection with the MTB. Post infection, the media is augmented with additional iron (40 to 80 μ M) as either ferric malate or FeCl₃. Numerous other cell types internalize bacteria by endocytosis or more specifically phagocytosis; endosymbionts or parasites have their own methods for cellular entry and these

natural processes can be exploited for internalization of the artificial endosymbionts resulting in the generation of so-called symbiosomes. In another embodiment, symbiosomes from one cell can be transplanted to another cell type (i.e., one incapable of endocytosis of artificial endosymbionts) using microinjection, organelle transplantation, and chimera techniques. These host cells are cultured in typical media and with the techniques for the specific cell type.

In one embodiment, the artificial endosymbiont is introduced to the host cell by a liposome mediated process. Mitochondria and chloroplasts, which are larger than MTB, have been efficiently introduced into eukaryotic cells when encapsulated into liposomes. (Bonnett, H. T. *Planta* 131, 229 (1976); Giles, K.; Vaughan, V.; Ranch, J.; Emery, J. Liposome-mediated uptake of chloroplasts by plant protoplasts. *In Vitro Cellular & Developmental Biology—Plant* 16(7) 581-584 (each of these two publications is incorporated by reference in its entirety for all purposes)). Numerous liposome fusion protocols and agents are available and can be used by the skilled artisan without undue experimentation. (See, e.g., Ben-Haim, N., Broz, P., Marsch, S., Meier, W., Hunziker, P. Cell-specific integration of artificial organelles based on functionalized polymer vesicles. *Nano Lett.* 8(5): 1368-1373 (2008); Lian, W., Chang, C., Chen, Y., Dao, R., Luo, Y., Chien, J., Hsieh, S., Lin, C. Intracellular delivery can be achieved by bombarding cells or tissues with accelerated molecules or bacteria without the need for carrier particles. *Experimental Cell Research* 313(1): 53-64 (2007); Heng, B. C. & Cao, T. Immunoliposome-mediated delivery of neomycin phosphotransferase for the lineage-specific selection of differentiated/committed stem cell progenies: Potential advantages over transfection with marker genes, fluorescence-activated and magnetic affinity cell-sorting. *Med Hypotheses* 65(2): 334-336 (2005); Potrykus (1990) Ciba Found Symp, Vol. 1 54: 198 (each of these four publications is incorporated by reference in its entirety for all purposes)).

The inventions disclosed herein will be better understood from the experimental details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the inventions as described more fully in the claims which follow thereafter.

EXAMPLES

Example 1

Microinjection of Gfp⁺AMB into Murine Cells

A. Construction of gfp⁺AMB.

Expression vectors for eGFP, one including a Shine-Dalgarno sequence upstream of the gfp gene and one without a Shine Dalgarno, sequence were cloned into cryptic broad host range vector pBBR1MCS-2 (Kovach, M. E., et al. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* 166, 175-176, (1995) (which is incorporated by reference in its entirety for all purposes)). AMB (ATCC 700264) was transformed with this construct. (Matsunaga, T. et al. Complete genome sequence of the facultative anaerobic magnetotactic bacterium *Magnetospirillum* sp. strain AMB-1. *DNA Res.* 12, 157-166 (2005); Burgess J. G., et al. Evolutionary relationships among *Magnetospirillum* strains inferred from phylogenetic analysis of 16S rDNA sequences. *J. Bacteriol.* 175: 6689-6694 (1993); Matsunaga T, et al. Gene transfer in magnetic bacteria: transposon mutagenesis and cloning of genomic DNA fragments

required for magnetosome synthesis. *J. Bacteriol.* 174: 2748-2753 (1992); Kawaguchi R, et al. Phylogeny and 16S rRNA sequence of *Magnetospirillum* sp. AMB-1, an aerobic magnetic bacterium. *Nucleic Acids Res.* 20: 1140, (1992) (each of these four publications is incorporated by reference in its entirety for all purposes)).

Transformation was achieved by conjugation using a donor *Escherichia coli* strain as described by Goulian, M. van der Woude, M. A. A simple system for converting lacZ to gfp reporter fusions in diverse bacteria. *Gene* 372, 219-226 (2006); Scheffel, A. Schüler, D. The Acidic Repetitive Domain of the *Magnetospirillum gryphiswaldense* MamJ Protein Displays Hypervariability but Is Not Required for Magnetosome Chain Assembly. *J. Bacteriol.* September; 189(17): 6437-6446 (2007) (each of these two publications is incorporated by reference in its entirety for all purposes). The mating reactions were cultured for 10 days under defined microaerophilic conditions in the absence of DAP to select for positive transformants.

Following conjugation, gfp⁺AMB transformants with and without the Shine-Dalgarno sequence successfully displayed GFP fluorescence. The transformants containing the Shine-Dalgarno sequence displayed higher levels of GFP fluorescence than the transformance without this sequence. The resulting fluorescence did not leave the gfp⁺AMB cells when viewed at 100× magnification at 488 nm excitation.

The magnetic properties of the gfp⁺AMB were analyzed by MRI. The gfp⁺AMB was suspended in agar plugs using a 1.5 T instrument to optimize and characterize the imaging properties. FIG. 1 shows the positive contrast generated with a T₁ pulse sequence over a log scale concentration up to ~10⁸ MTB/mL. Signal intensity was related to concentration.

B. Microinjection into Murine Embryonic Cells.

The gfp⁺AMB was microinjected into one cell of each of 170 mouse embryos at the 2-cell stage. Six concentrations over a log scale up to ~10⁵ gfp⁺AMB were injected per cell, estimated by the optical density at 565 nm. Death rate of cells following microinjection was constant across the different injected concentrations. Images overlaying fluorescent and differential interference contrast (DIC) images of cells injected with the highest concentration (10⁵ MTB/cell) were compared. An uninjected control exhibited low levels of autofluorescence. Slices at different horizontal planes in 8-cell embryos at a given time point were compared. In each embryo, all four cells derived from the injected cell showed significant fluorescence while none of the four cells derived from the uninjected internal controls displayed significant fluorescence.

The embryos were allowed to develop for three days after the injection. In each concentration level, embryos survived for up to the full three days developing to the 256 cell blastula stage and appeared healthy enough for implantation. Numerous cells within each blastula displayed significant fluorescence, demonstrating that the artificial endosymbionts were transferred to daughter cells across multiple cell divisions as the embryos comprising the eukaryotic host cells developed to the blastula stage. One such blastula is shown in FIG. 2, where Panel A shows a differential interference contrast (DIC) image of the blastula and Panel B) shows a gray scale fluorescence capture of the same image, showing fluorescence in numerous cells throughout the blastula.

Confocal microscopy was used to quantify total expression of GFP throughout four individual embryos by measuring total GFP fluorescence in the entire embryo over time at various points beginning at the eight cell stage of the embryo. FIG. 3 shows the change of embryo fluorescence over time. This indicates that the copy number of artificial endosymbionts was maintained in daughter cells for at least

seven generations, such that the fluorescent phenotype of the host cells was maintained as the embryo progressed from the 2-cell stage to the 256-cell blastula stage.

These results demonstrate that, when delivered by microinjection, gfp⁺AMB were not immediately cleared or degraded and were not toxic to the developing embryo over the course of the three day experiment. Microinjected embryos divided normally, suggesting that gfp⁺AMB do not display pathogenic markers or secrete toxic compounds. They were transferred to daughter cells across many cell divisions, were contained in the cytoplasm, were punctate and well distributed, and maintained copy number within the daughter host cells, such that the fluorescent phenotype of the eukaryote host cells was maintained in daughter cells through at least seven generations. These results demonstrate that AMB can be stably maintained intracellularly and are transferred to daughter cells over at least seven cell divisions.

Example 2

Phagocytic Entry of AMB

Receptor mediated: The inlAB gene is amplified from *L. monocytogenes* genomic DNA (ATCC 11911D-5) and is inserted into pBBR1MCS-5, 107 the gentamicin cognate of pBBR1MCS-2 (Kovach, M. E., et al. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* 166, 175-176, (1995)), and gfp+inlAB+ AMB is generated. The gfp+inlAB+ AMB is co-cultured with eukaryotic host cells, including common epithelial tumor cell lines Coco-2, MDA-MB231 and MCF7, non-epithelial tumor cell lines, such as HT-1080 and HL60, and murine stem cells. Fluorescent microscopy and FACS are used to monitor and quantify internalization and intracellular location.

Expression of pore-forming haemolysin (hlyA) in AMB is achieved through amplification of hlyA from *L. monocytogenes* genomic DNA (ATCC 11911D-5). The amplified hlyA is inserted into pBBR1MCS-3 (the tetracycline cognate of pBBR1MCS-2) which is then used to transform gfp⁺AMB. The resulting AMB strain is exposed to murine macrophage cell line J774, capable of spontaneous phagocytosis. Gentomycin treatment is used to eliminate bacteria not internalized and hlyA⁻AMB is used as negative control. Fluorescent microscopy is used to monitor the intracellular fate and localization of AMB.

If bacteria remain confined to the phagosomes, we will introduce two genes, plcA and plcB, implicated in escape of *L. monocytogenes* into the cytosol. (Smith, G. A., Marquis, H., Jones, S., Johnston, N. C., Portnoy, D. A., Goldfine, H. *Infection and immunity* 63: 4231 (1995); Camilli, A.; Goldfine, H.; Portnoy, D. A. *The Journal of Experimental Medicine* 173: 751 (1991) (each of these two publications is incorporated by reference in its entirety for all purposes)). If bacteria escape successfully, but fail to propagate, we will introduce hpt. (Goetz, M., Bubert, A., Wang, G., Chico-Calero, I., Vazquez-Boland, J. A., Beck, M.; Slaghuis, J., Szalay, A. A., Goebel, W. *Proc Natl Acad Sci USA* 98: 12221 (2001); Chico-Calero, I., Suarez, M., Gonzalez-Zorn, B., Scortti, M., Slaghuis, J., Goebel, W., Vazquez-Boland, J. A. *Proc Natl Acad Sci USA* 99: 431 (2002) (each of these two publications is incorporated by reference in its entirety for all purposes)). In *L. monocytogenes*, hpt encodes the transporter responsible for uptake of glucose-6-phosphate from the cytosol. Other genes from *L. monocytogenes* have been implicated in sustaining growth within host (glnA and gltAB and argD) and we will systematically introduce these as needed. (Joseph, B., Przybilla, K., Stuhler, C., Schauer, K.,

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Slaghuis, J., Fuchs, T. M., Goebel, W. *Journal of Bacteriol.* 188: 556 (2006) (which is incorporated by reference in its entirety for all purposes)).

Example 3

Regulation of AMB Growth

Regulation of AMB growth in embryonic stem cells can be regulated as follows. Coleoptericin-A (ColA) is amplified from total *Sitophilus oryzae* cDNA. Expression of ColA in beetles of genus *Sitophilus* regulates titers of γ -Proteobacterium, which has naturally developed close symbiotic relationship the beetles, and resides in specific cells called bacteriocytes. (Login, F. H., Balmain, S., Vallier, A., Vincent-Monegat, C., Vigneron, A., Weiss-Gayet, M., Rochat, D., Heddi, A. Antimicrobial peptides keep insect endosymbionts under control. *Science* 334(6054): 362-365 (2011) (which is incorporated by reference in its entirety for all purposes)).

Murine embryonic stem cells comprising gfp+AMB are treated using a neural differentiation protocol. MTB expression levels are quantified using qPCR and fluorescent microscopy. Amplified colA is then expressed in the gfp+AMB embryonic stem cells. A promoter is selected to provide optimal ColA expression levels.

All publications, patents and patent applications discussed and cited herein are incorporated herein by reference in their entireties. It is understood that the disclosed invention is not limited to the particular methodology, protocols and materials described as these can vary. It is also understood that the terminology used herein is for the purposes of describing particular embodiments only and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. A mammalian host cell comprising at least one magnetotactic bacterium, wherein the magnetotactic bacterium comprises a fluorescent reporter.

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2. The mammalian host cell of claim 1, wherein the fluorescent reporter is a GFP.

3. The mammalian host cell of claim 1, wherein the magnetotactic bacterium further comprising a pore-forming haemolysin encoded by a hlyA gene of *L. monocytogenes*.

4. The mammalian host cell of claim 1, wherein the mammalian host cell is a circulatory cell.

5. The mammalian host cell of claim 4, wherein the circulatory cell is a T-cell.

6. The mammalian host cell of claim 1, wherein the mammalian cell is a human cell.

7. The mammalian host cell of claim 1, wherein the mammalian cell is a murine cell.

8. The mammalian host cell of claim 1, wherein the mammalian cell is a cancer cell.

9. The mammalian host cell of claim 1, wherein the mammalian cell is a stem cell.

10. The mammalian host cell of claim 9, wherein the stem cell is a neural stem cell.

11. A method of detecting a mammalian cell comprising the steps of: obtaining the mammalian cell wherein the mammalian cell comprises a magnetotactic bacterium, and wherein the magnetotactic bacterium comprises a fluorescent reporter, and detecting the fluorescent reporter.

12. The method of claim 11, wherein the fluorescent reporter is a GFP.

13. The method of claim 11, wherein the magnetotactic bacterium further comprises a pore-forming haemolysin encoded by a hlyA gene of *L. monocytogenes*.

14. The method of claim 11, wherein the mammalian cell is a circulatory cell.

15. The method of claim 14, wherein the circulatory cell is a T-cell.

16. The method of claim 11, wherein the mammalian cell is a human cell.

17. The method of claim 11, wherein the mammalian cell is a murine cell.

18. The method of claim 11, wherein the mammalian cell is a cancer cell.

19. The method of claim 11, wherein the mammalian cell is a stem cell.

20. The method of claim 19, wherein the stem cell is a neural stem cell.

* * * * *